

In re Application of:
Tamburini et al.
Application No.: 09/974,026
Filed: October 10, 2001
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PATENT
Attorney Docket No.: AERO1130-4

IN THE SPECIFICATION

Please amend the following paragraphs in the specification as set forth below:

Please amend the paragraph beginning on page 6, line 27, and ending on page 6, line 35, as follows:

where the amino acid numbering corresponds to that of the amino acid sequence of native human placental bikunin. Another form of this domain can be a Kunitz-like domain consisting of the amino acid sequence of native human placental bikunin amino acids 106-156, "bikunin (106-156)" having the amino acid sequence:

CTANAVTGPCRASFP RWYFDVERN SCNNFIYGGCRGNKNSYRSEE 150

ACMLRC 156

~~(SEQ ID NO:7)~~ (SEQ ID NO:7)

Please amend the paragraph beginning on page 7, line 18, and ending on page 7, line 25, as follows:

An open reading frame which terminates at an early stop codon can still code for a functional protein. The instant invention encompasses such alternative termination, and in one embodiment provides for a protein of the amino acid sequence:

ADRERSIHDFCLVSKVVGR CRASMPRWWYNVTDGSCQLFVYGGCDGNSNN 50

YLTKEECLKKCATVTENATGDLATSRNAADSSVPSAPRRQDS 92

~~(SEQ ID NO:8)~~ (SEQ ID NO:8)

Please amend the paragraph beginning on page 8, line 25, and ending on page 8, line 30, as follows:

wherein sequence 1) is EST derived consensus SEQ ID NO:45, 2) is PCR clone SEQ ID NO:47, and 3) is lambda cDNA clone SEQ ID NO:49. In a preferred embodiment a protein of the instant invention comprises one of the amino acid sequences of SEQ ID NO: 45, or SEQ ID NO:47, or SEQ ID NO:49, wherein the protein has been cleaved in the region between the end of the last Kunitz domain and the transmembrane region.

Please amend the paragraph beginning on page 13, line 7, and ending on page 13, line 18, as follows:

Figure 3 depicts a deduced nucleic acid sequence of human placental bikunin (SEQ ID NO: 9) labeled "consensus" and matched with the translated protein amino acid sequence labeled "translated" (SEQ ID NO: 10). Also as comparison are shown the nucleic acid sequence for ESTs H94519 (SEQ ID NO: 16), N39798 (SEQ ID NO: 17), R74593 (SEQ ID NO: 14) and R35464 (SEQ ID NO: 12). The underlined nucleotides in the consensus sequence correspond to the site of PCR polymerase chain reaction (PCR) primers described in the Examples. Underlined amino acids in the translated consensus sequence are residues whose identity have been confirmed by amino acid sequencing of purified native human placental bikunin. Nucleotide and amino acid code are standard single letter code, "N" in the nucleic acid code indicates an unassigned nucleic acid, and "*" indicates a stop codon in the amino acid sequence.

Please amend the paragraph beginning on page 14, line 26, and ending on page 14, line 28, as follows:

Figure 7 depicts a silver stained SDS-PAGE gel sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of highly purified placental bikunin (lane 2), and a series of molecular size marker proteins (lane 1) of the indicated sizes in kilodaltons. Migration was from top to bottom.

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Please amend the paragraph beginning on page 55, line 20, and ending on page 55, line 31, as follows:

Identification of functional placental bikunin was achieved by measuring its ability to inhibit bovine trypsin and human plasma kallikrein. Trypsin inhibitory activity was performed in assay buffer (50 mM Hepes, pH 7.5, 0.1 M NaCl, 2.0 mM ~~CaCl₂~~ CaCl₂, 0.1% Triton x-100) at room temperature in a 96-well microtiter plate (Perkin Elmer) using Gly-Pro-Lys-Aminomethylcoumarin as a substrate. The amount of coumarin produced by trypsin was determined by measuring the fluorescence (ex = 370 nm, em = 432 nm) on a Perkin-Elmer LS-50B fluorimeter equipped with a plate reader. Trypsin (23 µg in 100 µl buffer) was mixed with 20 µl of the sample to be tested and incubated for 10 minutes at 25°C. The reaction was started by the addition of 50 µl of the substrate GPK-AMC (33 µM final concentration) in assay buffer. The fluorescence intensity was measured and the % inhibition for each fraction was determined by: